Sonic signals somites

Sonic hedgehog, a secreted signalling molecule known to play a role in the patterning of the central nervous system and the limb in vertebrates, also controls differentiation of the somites.

Although it has been known for decades that axial midline structures, such as the notochord and neural tube, influence patterning of the nearby paraxial mesoderm in vertebrates [1], the mechanisms underlying this phenomenon have remained obscure. Recent studies by two groups [2,3] have shed considerable light on this issue. They have demonstrated a role for the secreted protein, Sonic hedgehog, which is synthesized in the notochord and floor plate, in the determination of cell fate during somite differentiation. Previous work has established roles for Sonic hedgehog in dorsal–ventral patterning of the central nervous system and in anterior–posterior specification in the limb (reviewed in [4]). Now, the developmental repertoire of this factor is expanded to include determination of the paraxial mesoderm.

During vertebrate embryogenesis, the segmental plate mesoderm that lies adjacent to the neural tube in the trunk and tail becomes segmented into paired epithelial somites. Each transient somite then differentiates into the ventral sclerotome, from which the skeletal elements of the ribs and vertebral column derive, and the dorsal dermomyotome. The dermomyotome further differentiates into the dorsal–lateral dermotome, which gives rise to dermis, and the myotome, sandwiched between the sclerotome and the dermotome, which gives rise to the axial musculature (see Fig. 1).

Tissue transplantation experiments, primarily with chick embryos, have demonstrated that the development of the somites is controlled by axial structures [5]. Specifically, grafts of notochord or floor plate, placed dorsally, lead to suppression of dermomyotome development with concomitant expansion of the sclerotome. Conversely, removal of the notochord leads to a lack of sclerotome development, as do mouse mutations that interfere with notochord formation [6]. To ask whether Sonic hedgehog might mediate the effects of the notochord and floor plate on somite development, Johnson *et al.* [2] and Fan and Tessier-Lavigne [3] took distinct but complementary approaches. Both groups concluded that Sonic hedgehog indeed plays a major role in inducing the formation of the sclerotome ventrally while preventing dermomyotome formation in this region. In addition, Johnson *et al.* [2] suggest a role for Sonic hedgehog in the development of the myotome.

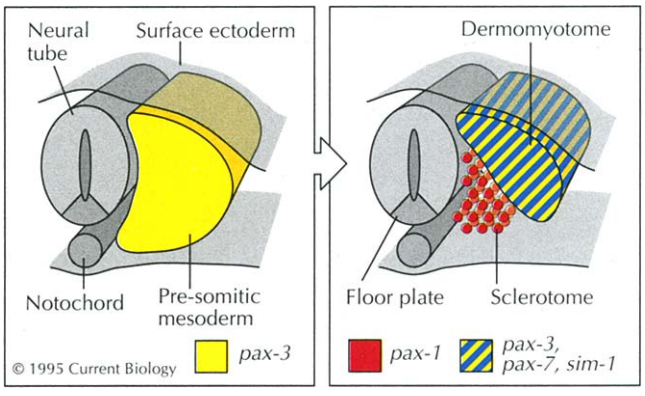

Fig. 1. Expression patterns of markers during early development of the somites.

In the chick, Sonic hedgehog is first expressed in Hensen's node (a putative organizing center) prior to formation of the first somite, and it continues to be expressed in the notochord and floor plate throughout somitogenesis, which proceeds by the condensation of successive somites in a caudal direction. Somites and presomitic mesoderm are therefore continuously exposed to a ventral source of Sonic hedgehog. To determine what role this factor might play in somite patterning *in vivo*, Johnson *et. al.* [2] ectopically expressed Sonic hedgehog in the dorsal segmental plate by infection with a retrovirus engineered to express the *sonic hedgehog* gene. Two days after infection, embryos were analyzed with molecular markers diagnostic for different somitic regions.

One such marker, the transcription factor-encoding gene *pax-1*, is first expressed in chick somites ventral–medially in the fourth most recently formed somite, at the time when dorsal–ventral pattern becomes irreversibly specified. As the cells in this region delaminate to form the sclerotome, *pax-1* expression intensifies and spreads laterally, forming a sharp border with the still-epithelial dermomyotome; *pax-1* is therefore a specific marker for sclerotomal cells and their precursors. Embryos injected with the Sonic-hedgehog-encoding virus show a dorsal expansion of the *pax-1* expression domain on the infected side. Thus, Sonic hedgehog may either reprogram dorsal cells to adopt a sclerotomal fate or increase proliferation specifically within the sclerotome, so expanding the *pax-1*-expressing population.

To determine whether reprogramming had indeed taken place, the expression of *pax-3* was assessed. This factor is normally expressed at a low level throughout the chick segmental plate, but is restricted to the dermomyotome and finally the dermotome as somite development proceeds. Ectopic expression of *sonic hedgehog* in the dorsal

mesoderm abolishes *pax-3* expression in the region of the presumptive dermotome, which is consistent with the respecification model, and mimics the effects of the notochord and floor plate in reprogramming somitic tissue.

Fan and Tessier-Lavigne [3] reached similar conclusions regarding the role of Sonic hedgehog in dorso-ventral patterning of the mouse somite. By combining presomitic mesoderm from mouse embryos with explants of chick notochord or ventral neural tube (including the floor plate), they confirmed the ability of these tissues to induce sclerotome, as revealed by the induction of *pax-1* expression. Cells expressing *pax-1* are found throughout the presomitic mesoderm explant over a distance of 150–200 μm. The ability of these tissues to induce *pax-1* expression even when separated from the responding tissue by a 25 μm-thick filter implies that a diffusible factor is involved. Induction of *pax-1* expression is nearly as efficient when the presomitic mesoderm is cultured next to a pellet of tissue-culture cells transfected with a *sonic hedgehog* expression construct. Again, induction is seen over a large distance and can occur through a filter. To rule out the possibility that the *pax-1* induction observed at a distance from the inducing tissue is an indirect effect of other factors, the authors showed that chick sclerotomal tissue already expressing *pax-1* is not itself able to induce *pax-1* expression in mouse presomitic mesoderm explants. Thus, there does not seem to be a relay of signals triggered by Sonic hedgehog; rather, the authors conclude [3] that the ability of notochord and floor plate explants to induce *pax-1* is a direct effect of a diffusible form of Sonic hedgehog.

The ability of notochord/floor plate explants or *sonic-hedgehog*-expressing tissue-culture cells to induce *pax-1* expression over such large distances *in vitro* raises a question: what prevents *pax-1* expression across the entire somite *in vivo*? The answer appears to be competitive signaling from the dorsal ectoderm and neural tube, which promotes dermomyotome development. Earlier work has highlighted the importance of the surface ectoderm in maintaining dermomyotomal gene expression [7]. Fan and Tessier-Lavigne [3] therefore used their tissue recombinant system to assay dorsal patterning signals from the surface ectoderm. They observed that when presomitic mesoderm is cultured in close contact with the basal surface of an ectoderm fragment, it expresses the dorsal-specific markers *pax-3*, *sim-1* and *pax-7* (Fig. 1). Although *pax-3* and *sim-1* are expressed at a low level throughout the presomitic mesoderm, their expression is lost within 24 hours when this tissue is cultured alone. The *pax-7* gene encodes a dermomyotome-specific factor expressed in more advanced somites. Unlike *pax-1* induction by Sonic-hedgehog-expressing cells or tissues, the induction of dorsal cells by the surface ectoderm is a local phenomenon. The *pax-3/sim-1/pax-7*-expressing cells are found near the boundary of the combined tissues. Also, no induction occurs through a filter, nor if the apical surface of the ectoderm is in contact with the mesoderm. Dermomyotome induction by the surface

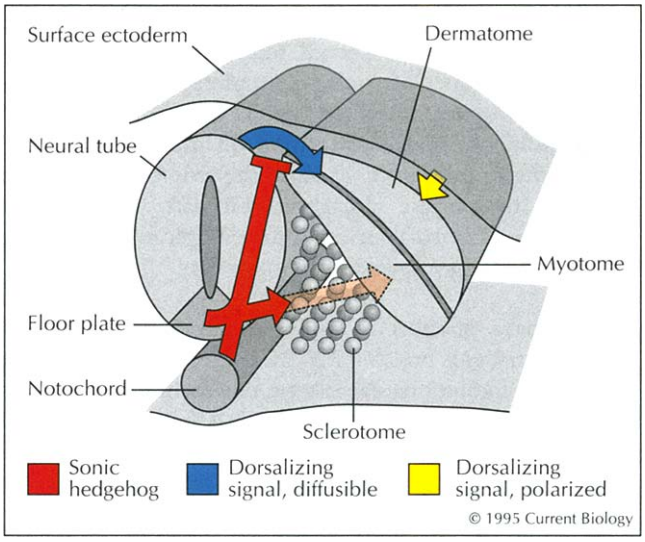

Fig. 2. Proposed signals regulating somite development. Sonic hedgehog from the notochord and floor plate induces sclerotome development (red arrow) and inhibits the dorsalizing signal (blue arrow) from the dorsal neural tube that acts on the dermatome and myotome. Inhibition is illustrated by the red line ending in a 'T'. Sonic hedgehog is also shown influencing the development of the myotome (broken arrow). Finally, a short-range polarized signal from the surface ectoderm acts on the dermatome and myotome (yellow arrow).

ectoderm therefore appears to involve a short-range polarized signal.

Interestingly, the dorsal neural tube is also able to induce *pax-3*, *sim-1* and *pax-7* over a large distance in presomitic mesoderm explants, even if the tissues are separated by a filter. This is at odds with the *in vivo* situation, in which these markers are not expressed in the mesoderm nearest the neural tube. Again, competitive signaling from the notochord and floor plate is invoked to explain this discrepancy. To demonstrate that competition occurs, Fan and Tessier-Lavigne [3] made triple recombinants, in which presomitic mesoderm simultaneously contacts dorsal neural tube and notochord (or *sonic-hedgehog*-expressing cells). In these cultures, dermomyotomal markers are induced, but only in the region of the presomitic mesoderm that is closest to the neural tube. The remaining tissue expresses the sclerotome marker, *pax-1*.

As notochord and dorsal neural tube are capable of inducing ventral and dorsal markers, respectively, throughout presomitic mesoderm explants, the results with the triple recombinants establish that competition between these two signaling tissues occurs. What, then, could be the role *in vivo* of a long-range dorsalizing signal emanating from the neural tube? Recent evidence suggests that such a signal may be necessary for myotome formation [8]. Indeed, it appears that signals from both the dorsal neural tube and the notochord/floor plate may be necessary for normal myotome formation. That the latter signal may be Sonic hedgehog is supported by the observation made by Johnson *et al.* [2] that ectopic dorsal expression of *sonic hedgehog* leads to overproliferation and

disorganization of the myotome. This is precisely the opposite of what is observed following a dorsal notochord/floor plate graft, which suppresses myotome formation. However, it could be a secondary effect of suppression of dorsal patterning in the neural tube, which might inhibit or abolish the putative myogenic inducer. In contrast, dorsal–ventral patterning of the neural tube is normal in embryos ectopically expressing *sonic hedgehog* dorsally after viral infection.

On the basis of what is known about Sonic hedgehog biochemistry, is it possible for this molecule to be a direct long-range inducer of sclerotome development, as well as a regulator of myotome formation? It has recently been demonstrated that both *Drosophila* Hedgehog and vertebrate Sonic hedgehog proteins undergo autocatalytic cleavage to yield secreted amino- (N) and carboxy-terminal (C) peptides [9,10]. Furthermore, it appears that all the signaling capacities of the *Drosophila* protein are contained within the highly conserved N peptide ([11], and [12] in this issue of *Current Biology*), and it is likely that this is true for the vertebrate protein as well [13]. But there is ample evidence that most of the N peptide does not diffuse appreciably from secreting cells, but rather is 'tethered' at or near the cell surface ([9–12] and E. Marti, R. Takada, D.A.B. and A.P.M., unpublished observations). Perhaps additional factors in the ventral neural tube, notochord or paraxial mesoderm can release the N peptide from the cells that synthesize it. If this is so, then the peptide should be detectable at a distance from the notochord and floor plate during early stages of somitogenesis. Of course, failure to detect Sonic hedgehog by immunological methods does not rule out long-range diffusion of a small proportion of the protein. If this is the case, then it must be demonstrated that such a low level of purified Sonic hedgehog protein is able to exert the same developmental effects on mesoderm explants as the notochord, floor plate and *sonic hedgehog*-expressing cells.

Ultimate proof that Sonic hedgehog is indeed a key regulator of somite patterning will require inactivation of the signal at key points during somite differentiation; gene targeting is likely to be the most informative approach to this end. Clearly, there are still important questions to be answered concerning Sonic hedgehog's role in somite patterning, particularly in the dose requirement for distinct inductive interactions. Given the rapid progress made so far in exploring this interesting molecule, the answers should not be far away.

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